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## RESEARCH ARTICLE

# The effect of *Banisteriopsis caapi* (*B. caapi*) on the motor deficits in the MPTP-treated common marmoset model of Parkinson's disease

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*Banisteriopsis caapi* (*B. caapi*) contains harmine, harmaline, and tetrahydroharmine, has monoamine oxidase inhibitory activity, and has reported antiparkinsonian activity in humans when imbibed as a tea; however, its effects are poorly documented. For this reason, motor function was assessed in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated common marmosets following administration of *B. caapi* extract (28.4–113.6 mg/kg; po), harmine (0.1 and 0.3 mg/kg; sc), and selegiline (10 mg/kg; sc), alone or with a submaximal dose of L-3,4-dihydroxyphenylalanine (L-DOPA; 4–7 mg/kg). L-DOPA reversed motor disability, increased locomotor activity, and induced moderate dyskinesia. *B. caapi* did not increase locomotor activity or induce dyskinesia but at 56.8 and 113.6 mg/kg improved motor disability. The L-DOPA response was unaltered by co-administration of *B. caapi*. Harmine (0.1 and 0.3 mg/kg) produced a mild improvement in motor disability without affecting locomotor activity or dyskinesia but had no effect on the L-DOPA-induced antiparkinsonian response. Selegiline (10 mg/kg) alone improved motor function to the same extent as L-DOPA, but with only mild dyskinesia, and did not alter the response to L-DOPA, although dyskinesia was reduced. The findings suggest that *B. caapi* alone has a mild antiparkinsonian effect but does not enhance the L-DOPA response or reduce dyskinesia.

## KEYWORDS

*Banisteriopsis*, monoamine oxidase, MPTP, Parkinson's disease, primate

## 1 | INTRODUCTION

Parkinson's disease (PD) is a progressive, neurodegenerative disease that affects motor function resulting primarily from the degeneration of dopaminergic neurons in the substantia nigra pars compacta. Treatment is based on dopamine replacement therapy, and the amino acid precursor of dopamine, L-3,4-dihydroxyphenylalanine (L-DOPA), is the most widely used and efficacious symptomatic treatment; however, chronic use commonly leads to a loss of efficacy and the development of unwanted involuntary movements (dyskinesia; Ahlskog & Muentner, 2001; Fox & Lang, 2008). To extend the duration of effect of L-DOPA and reduce the dose required, monoamine oxidase B (MAO-B) inhibitors, such as selegiline and

rasagiline, are employed to inhibit the metabolism of dopamine derived from L-DOPA (Jenner, 2015).

*Banisteriopsis caapi* (*B. caapi*) is a liana, endemic to the Amazon, basin known to contain a number of  $\beta$ -carbolines such as harmine, harmaline, and tetrahydroharmine that possess MAO inhibitory properties (Schwarz, Houghton, Rose, Jenner, & Lees, 2003). Extracts of *B. caapi* have been shown to be effective in treating PD when used in combination with L-DOPA by extending its duration of action possibly as a result of reduced dopamine metabolism and enhanced release of dopamine from dopaminergic neurons (Serrano-Duenas, Cardozo-Pelaez, & Sanchez-Ramos, 2001a, 2001b). It is thought that these effects of *B. caapi* are mediated through its constituent  $\beta$ -carbolines (Iurlo et al., 2001; Meneguz, Betto, & Ricciarello, 1994). Indeed,

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90 years ago, Lewin and Beringer both reported the successful use of harmine as monotherapy in the pre-L-DOPA era for the treatment of PD and although marketed in the 1920s for this purpose, it fell out of use because of low efficacy (see Djamshidian, Bernschneider-Reif, Poewe, & Lees, 2016).

More recently, Meneguz et al. (1994) reported that short-term intravenous administration of harmine used in combination with L-DOPA increased its plasma concentration and raised dopamine levels in the striatum of rats and rabbits (Iurlo et al., 2001; Meneguz et al., 1994). This supported the idea that MAO inhibition by *B. caapi* extracts underlies the antiparkinsonian activity observed, but this has never been formally tested.

The purpose of this study was to determine the effects of *B. caapi* extracts and one of its constituent  $\beta$ -carbolines, harmine, on motor dysfunction in a primate model of PD. To that end, we have used the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated common marmoset primed by previous chronic treatment with L-DOPA to exhibit dyskinesia, an established model of the motor symptoms of PD. The treatments were evaluated both as monotherapy and in combination with L-DOPA and compared with the established MAO-B inhibitor, selegiline.

## 2 | MATERIALS AND METHODS

### 2.1 | Preparation of extract of *B. caapi*

Dried stem of *B. caapi* was obtained from Dr. Marcos Serrano-Duenas, Neurology Service, Carlos Andrade Marin Hospital Quito. An extract from the dried whole stem of *B. caapi* was prepared as previously described (Schwarz et al., 2003), approximating to the method of preparation of the extract used in Ecuador for the earlier clinical trials in PD. The stem was ground into a powder and then refluxed (50 g/500 ml distilled water) for 1 hr. After filtration, the remaining plant material was refluxed (50 g/500 ml distilled water) for 1 hr as before and filtered, and the two filtrates were combined and freeze-dried. The freeze-dried aqueous extracts were then reconstituted in methanol and analysed by high-performance liquid chromatography (HPLC) with diode array for levels of harmine, harmaline, and tetrahydroharmine.

### 2.2 | HPLC analysis

The samples were analysed on a HP1050 HPLC system equipped with an autosampler, a quaternary pump, and a diode-array detector. A Zorbax SB C-18 2.1 mm  $\times$  10 cm (particle size 3.5 micron) column was employed. A linear gradient of mobile Phase B (acetonitrile containing 0.1% trifluoroacetic acid) over mobile Phase A (0.1% trifluoroacetic acid in water) from 0% to 50% B in 50 min was employed at a flow rate of 0.2 ml/min, and the eluents were monitored at wavelengths between 210 and 280 nm. Data were collected and compared with standard curves harmine, harmaline, tetrahydroharmine, and N,N-dimethyltryptamine using ChemStation software.

### 2.3 | Animals

All studies were performed in the same group ( $n = 8$ ) of previously MPTP-treated common marmosets (*Callithrix jacchus*; aged 3–10 years; Pearce, Jackson, Smith, Jenner, & Marsden, 1995; Smith et al., 2000; Zubair et al., 2007). Animals were housed in mixed sex or female–female pairs under standard housing conditions (23 °C, humidity 50%). They have access to water and marmoset pellets ad libitum and received meals of fresh fruit, cereals, and protein (e.g., egg and chicken) twice daily. Vitamin D supplement is given once per week.

Animals were treated with MPTP between 1 and 4 years prior to the start of the study (Smith, Jackson, Hansard, Maratos, & Jenner, 2003). Following MPTP treatment, the animals became bradykinetic and rigid and some showed action tremor. They exhibited reduced vocalization; however, they were able to eat, drink, and move around the home cage unaided. All animals were responsive to L-DOPA and had been primed with L-DOPA (8–12.5 mg/kg po + benserazide (10 mg/kg po) for up to 28 days) to express dyskinesia between 1 and 4 years prior to these studies.

All experiments were performed according to the Animals (Scientific Procedures Act) 1986 under Project Licence No 70/7146, with local ethical approval. All animals had previously been involved in studies involving the assessment of novel drugs for PD and received a drug washout period of at least 4 weeks prior to the start of this study. Drug treatments were performed according to a modified Latin square schedule such that all animals were given all treatments on a single occasion in a randomized manner. Experiments were performed twice weekly with at least 72 hr between treatments to ensure appropriate animal welfare consideration.

### 2.4 | Treatments

Benserazide is a DOPA-decarboxylase inhibitor and prevents the peripheral conversion of L-DOPA to dopamine. Benserazide HCl (10 mg/kg, Sigma, UK) was dissolved in 10% sucrose and then used as the vehicle for dissolving L-DOPA methyl ester (ME) such that benserazide and L-DOPA-ME were then dosed concomitantly in the same syringe.

L-DOPA-ME (Sigma, UK) was used throughout this study as it is easily soluble and rapidly hydrolysed to the parent compound and produces the same effect as using the freebase (Brunnergeunat et al., 1995). L-DOPA-ME was dissolved in 10% sucrose (containing benserazide as above) using a salt:base ratio of 1:1.25 to provide the freebase dose (L-DOPA 4 or 7 mg/kg). All doses were corrected for freebase and referred to as L-DOPA.

L-DOPA was administered at a submaximal dose determined from dose ranging studies (data not shown) and performed in the animals immediately prior to the present studies. The dose volume was 2 ml/kg administered by oral gavage (po).

Domperidone HCl (2 mg/kg, Sigma, UK) was prepared as a suspension in 10% sucrose and administered orally at a dose volume of 2 ml/kg, 1 hr prior to treatment to prevent vomiting.

*B. caapi* was analysed for content of harmine, harmaline, and tetrahydroharmine, which are known to be the three main chemicals in this plant. The stem extracts contained 35.2 mg/g extract of

harmine, 17.6 mg/g extract of tetrahydroharmine, and no harmaline (Figure 1a,b). *B. caapi* extract (28.4, 56.8, or 113.6 mg/kg) was dissolved in 20% Kleptose HPB (hydroxypropyl-beta-cyclodextrin-HPBCD, Roquette pharma, France) and administered by oral gavage (po) at a dose volume of 2 ml/kg.

Harmine HCL (Santa Cruz Biotechnology, Germany) was dissolved in 0.9% sterile saline (Baxter Healthcare Ltd., UK) using a salt: base ratio of 1:1.17 to provide the freebase dose (Harmine 0.1 and 0.3 mg/kg). The dose volume was 1 ml/kg administered by subcutaneous injection (sc).

Selegiline HCl (Sigma, UK) was dissolved in 0.9% sterile saline (Baxter Healthcare Ltd., UK) using a salt base ratio of 1:1.195 to provide the freebase dose of selegiline (10 mg/kg). The dose volume was 1 ml/kg administered by subcutaneous injection (sc).

## 2.5 | Administration of *B. caapi* extract

Animals were treated with *B. caapi* extract (28.4, 56.8, or 113.6 mg/kg po) or vehicle (20% Kleptose po) in combination with a submaximal dose of L-DOPA (7 mg/kg po) or vehicle (10% sucrose po).

Initially a dose of *B. caapi* extract (8.5 mg/2 ml po), equivalent to harmine (0.3 mg/kg), was given to all animals in their home cage. No

changes in activity or disability were noted (data not shown). Importantly, the animals drank the concentrated tea with no adverse effect. Therefore, the lowest dose of *B. caapi* extract chosen (28.4 mg/kg) was equivalent to drinking 2.36 ml/kg of the original tea and contained the equivalent of 1 mg/2 ml harmine.

On the day of the study, *B. caapi* extract or vehicle was administered immediately prior to L-DOPA (7 mg/kg, po) or vehicle at  $t = 0$  hr. All drug combinations were administered in a randomized manner according to a modified Latin square such that all animals received all treatments with at least 2 days between each treatment.

## 2.6 | Administration of harmine

Animals were treated with harmine (0.1 and 0.3 mg/kg, sc) or vehicle (0.9% saline sc) in combination with a submaximal dose of L-DOPA (4 mg/kg, po) or vehicle (10% sucrose po). Harmine or vehicle was administered immediately prior to administration of L-DOPA (4 mg/kg, po) or vehicle. Drugs were administered in a randomized manner according to a modified Latin square such that all animals received all drug combinations with at least 2 days between each treatment.

## 2.7 | Administration of selegiline

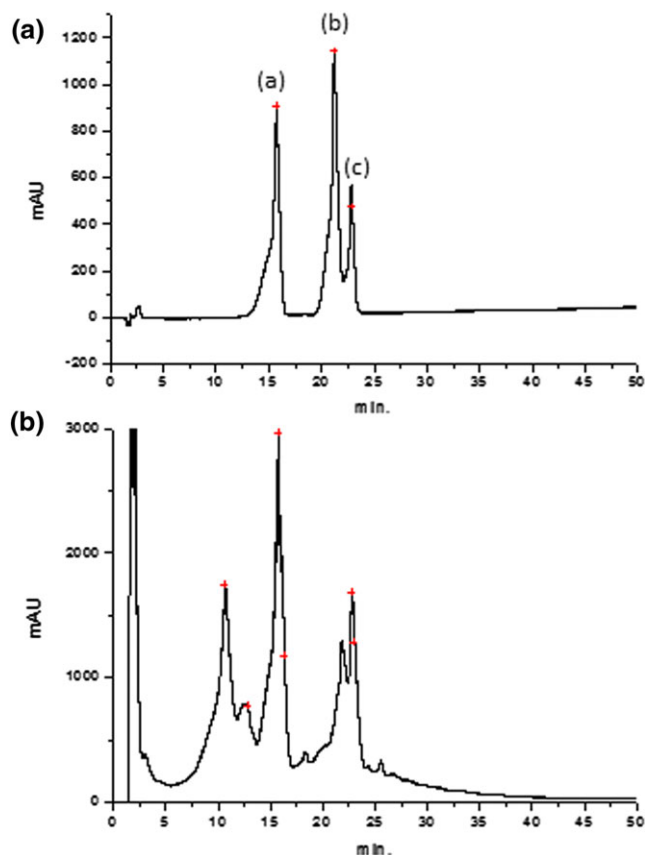
The effect of selegiline (10 mg/kg, sc) on basal and L-DOPA (4 mg/kg, po)-induced motor activity was investigated as positive control in a separate experiment. Animals were treated with either selegiline (10 mg/kg sc) plus vehicle (10% sucrose po) or selegiline (10 mg/kg sc) plus a submaximal dose of L-DOPA (4 mg/kg, po). Selegiline was administered 60 min prior to L-DOPA (4 mg/kg, po) or vehicle at  $t = -60$  min. Treatments were administered in a crossover design with 1 week between each treatment to allow washout of drug due to the long half-life of selegiline.

## 2.8 | Behavioural assessment

On test days, the animals were removed from their home cages before presentation of breakfast and were placed individually into automated test units (Smith et al., 2002). They were allowed a 60-min acclimatization period prior to treatment during which basal locomotor activity, motor disability, and dyskinesia were assessed before (baseline) and for up to a maximum of 6 hr as described below.

## 2.9 | Locomotor activity

Each automated test unit (50 × 60 × 90 cm) was fitted with a clear Perspex door to facilitate observation and equipped with eight horizontally orientated photoelectric emitters/detectors (light beams), arranged to permit optimal assessment of activity. Interruption of a light beam was automatically recorded as a single locomotor count accumulated in 10- and 30-min intervals for 5 hr following drug treatment.



**FIGURE 1** High-performance liquid chromatography (HPLC) traces of tetrahydroharmine, harmine, and harmaline in the reconstituted freeze-dried aqueous extract of *Banisteriopsis caapi*. The extract from the dried whole stem of *B. caapi* was analysed by HPLC with diode array at wavelengths between 210 and 280 nm for levels of (a) tetrahydroharmine, (b) harmine, and (c) harmaline. HPLC traces from (a) standard containing 0.3 mg/ml of all alkaloids and (b) original *B. caapi* extract [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

## 2.10 | Motor disability

Motor disability was assessed simultaneously with locomotor activity through a one-way mirror by experienced observers blinded to treatment. Basal disability was assessed before and once every 30 min after drug treatment for 5 hr using an established motor disability rating scale; alertness (normal = 0, reduced = 1, sleepy = 2); checking (present = 0, reduced = 1, absent = 2); posture (normal = 0, abnormal trunk +1, abnormal tail +1, abnormal limbs +1, flexed = 4); balance (normal = 0, impaired = 1, unstable = 2, spontaneous falls = 3); reaction to stimuli (normal = 0, reduced = 1, slow = 2, absent = 3); vocalization (normal = 0, reduced = 1, absent = 2); motility (normal = 0, bradykinesia = 1, akinesia = 2). These values were summed, a maximum score of 18 indicating severe motor disability, a minimum score of 0 indicating maximum reversal of motor disability.

## 2.11 | Dyskinesia

Dyskinesia was assessed simultaneously with motor disability by experienced observers blinded to treatment. Basal dyskinesia was assessed before and once every 30 min after drug treatment for 5 hr using an established dyskinesia rating scale; 0 = *absent*; 1 = *mild, fleeting, and rare dyskinetic postures and movements*; 2 = *moderate: more prominent abnormal movements, but not significantly affecting normal behaviour*; 3 = *marked, frequent and at times continuous dyskinesia affecting the normal pattern of activity*; 4 = *severe, virtually continuous dyskinetic activity, disabling to the animal and replacing normal behaviour*.

## 2.12 | Data and statistical analysis

Data are presented as median in time course and area under curve (AUC) (calculated over 5 hr using the trapezoid method from the time course curves, Graphpad Prism®).

Motor disability and dyskinesia scores were transformed using the formula  $Y = \sqrt{Y}$  prior to statistical analysis. Changes in the AUC for locomotor activity, reversal of motor disability, and dyskinesia expression were then analysed using a repeated measures one-way analysis of variance (ANOVA) and Newman-Keuls test. Significance was set at  $p < .05$  for all analyses and performed using GraphPad Prism Version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

# 3 | RESULTS

## 3.1 | The effect of *B. caapi* alone and combined with L-DOPA (7 mg/kg, po) on locomotor activity, motor disability, and dyskinesia

Neither vehicle treatments (Kleptose or sucrose) had any effect on motor disability, locomotor activity and did not induce dyskinesia (Figure 2a–f); however, the submaximal dose of L-DOPA (7 mg/kg, po) produced a significant reversal of motor disability, an increase in locomotor activity and induced moderate to severe dyskinesia. *B. caapi* improved overall motor disability scores with a U-shaped dose response curve, with a maximum, but moderate reversal of motor

disability following 56.8 mg/kg. This reversal was significantly different to vehicle following both 56.8 and 113.6 mg/kg *B. caapi* alone (Figure 2b). No one aspect of motor disability was specifically affected by *B. caapi* treatment. By contrast, at all doses investigated (28.4 to 113.6 mg/kg), *B. caapi* alone had no effect on locomotor activity and did not induce dyskinesia (Figures 2c–f). When given in combination with a submaximal dose of L-DOPA (7 mg/kg, po), *B. caapi* produced no significant alteration to the L-DOPA (7 mg/kg, po) response, although L-DOPA (7 mg/kg, po)-induced locomotor activity was somewhat reduced with increasing dose of *B. caapi*, but this was not statistically significant (Figure 2d). The lowest dose of *B. caapi* (28.4 mg/kg) caused a slight reduction in L-DOPA (7 mg/kg, po)-induced total dyskinesia score; however, this reduction was not statistically different when compared with L-DOPA (7 mg/kg, po) alone (Figure 2f).

## 3.2 | The effect of treatment with harmine alone and in combination with L-DOPA (4 mg/kg, po) on locomotor activity, motor disability, and dyskinesia

As expected, the submaximal dose of L-DOPA (4 mg/kg, po) produced a significant reversal of motor disability, an increase in locomotor activity, and expression of mild to severe dyskinesia (Figure 3a–f). The higher dose of harmine (0.3 mg/kg sc) alone produced a small reversal of motor disability that lasted for about 2 hr with total scores significantly improved compared with vehicle (Figure 3b). By contrast, harmine had no effect on locomotor activity at any dose given alone (Figure 3c,d). Although three animals showed a fleeting display of abnormal movements, the dyskinesia scores were also not significantly different to control (Figures 3e,f).

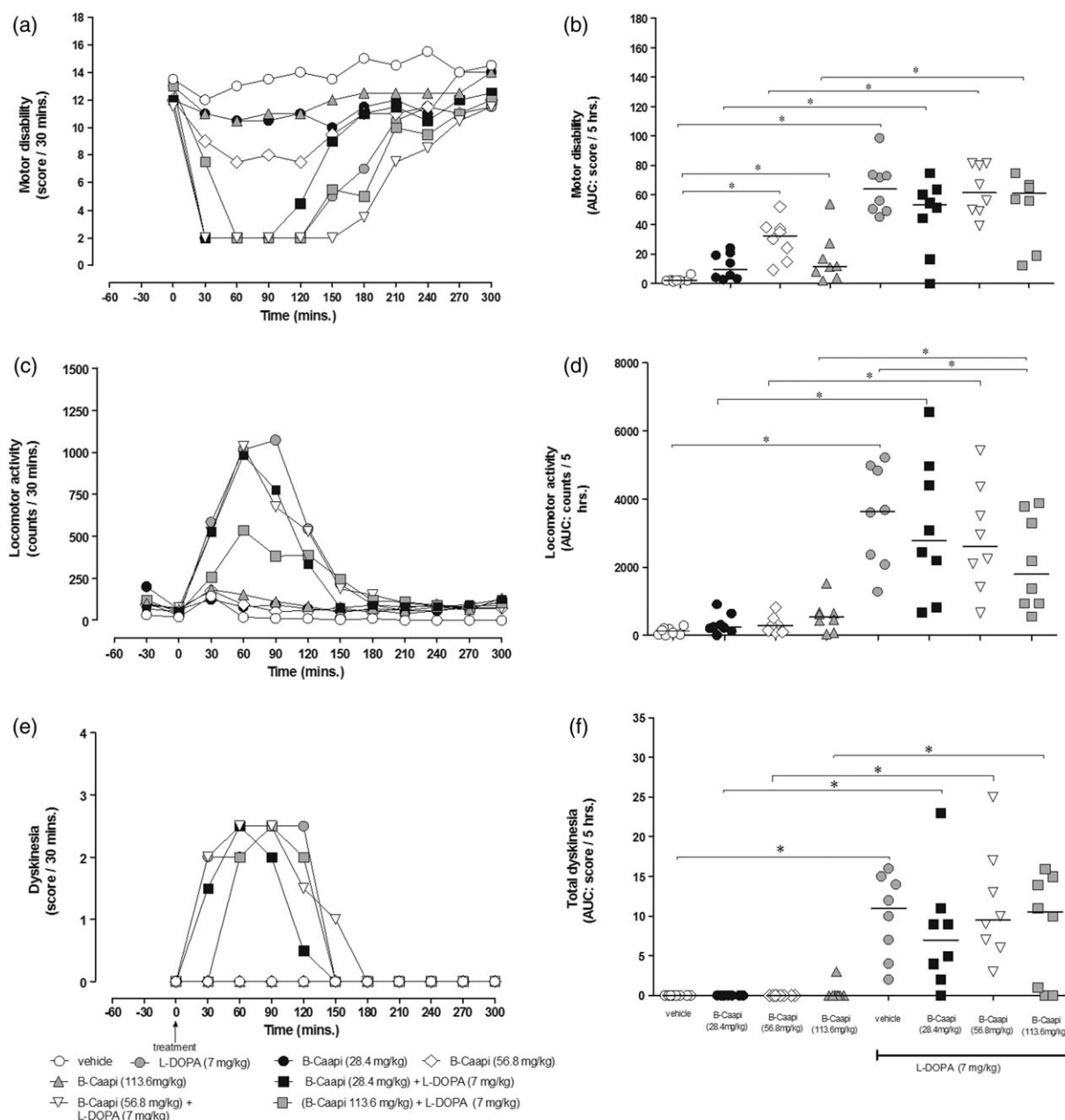
L-DOPA (4 mg/kg, po)-induced reversal of motor disability or increase in locomotor activity was not altered by any dose of harmine (0.1 and 0.3 mg/kg sc) (Figures 3a–d).

The severity of L-DOPA (4 mg/kg, po)-induced peak dose dyskinesia seen 1 hr after dosing tended to be increased by harmine (0.1 and 0.3 mg/kg; Figure 3e,f), although the scores were not significantly different to L-DOPA (4 mg/kg, po) alone. No one individual component of the motor disability scores was specifically altered by harmine except in two animals where existing action tremor was worsened.

## 3.3 | The effect of treatment with selegiline alone and in combination with L-DOPA (4 mg/kg, po) on locomotor activity, motor disability, and dyskinesia

Selegiline (10 mg/kg, sc) alone produced a similar reversal of motor disability and increase in locomotor activity as the submaximal dose of L-DOPA (4 mg/kg, po), although with a reduced peak effect. Motor disability was significantly improved compared with vehicle (Figures 4a–d). Overall motor disability reversal and locomotor activity induced by L-DOPA (4 mg/kg, po) was not altered by selegiline (Figures 4b,d); however, the duration of effect was increased with improved motor disability scores (score < 8) up to 210 min compared with 150 min with L-DOPA alone. Interestingly, when given alone, selegiline produced fleeting dyskinesia in some animals (scores 1–2), which did not persist but significantly reduced the L-DOPA (4 mg/kg, po)-induced dyskinesia (Figure 4f).



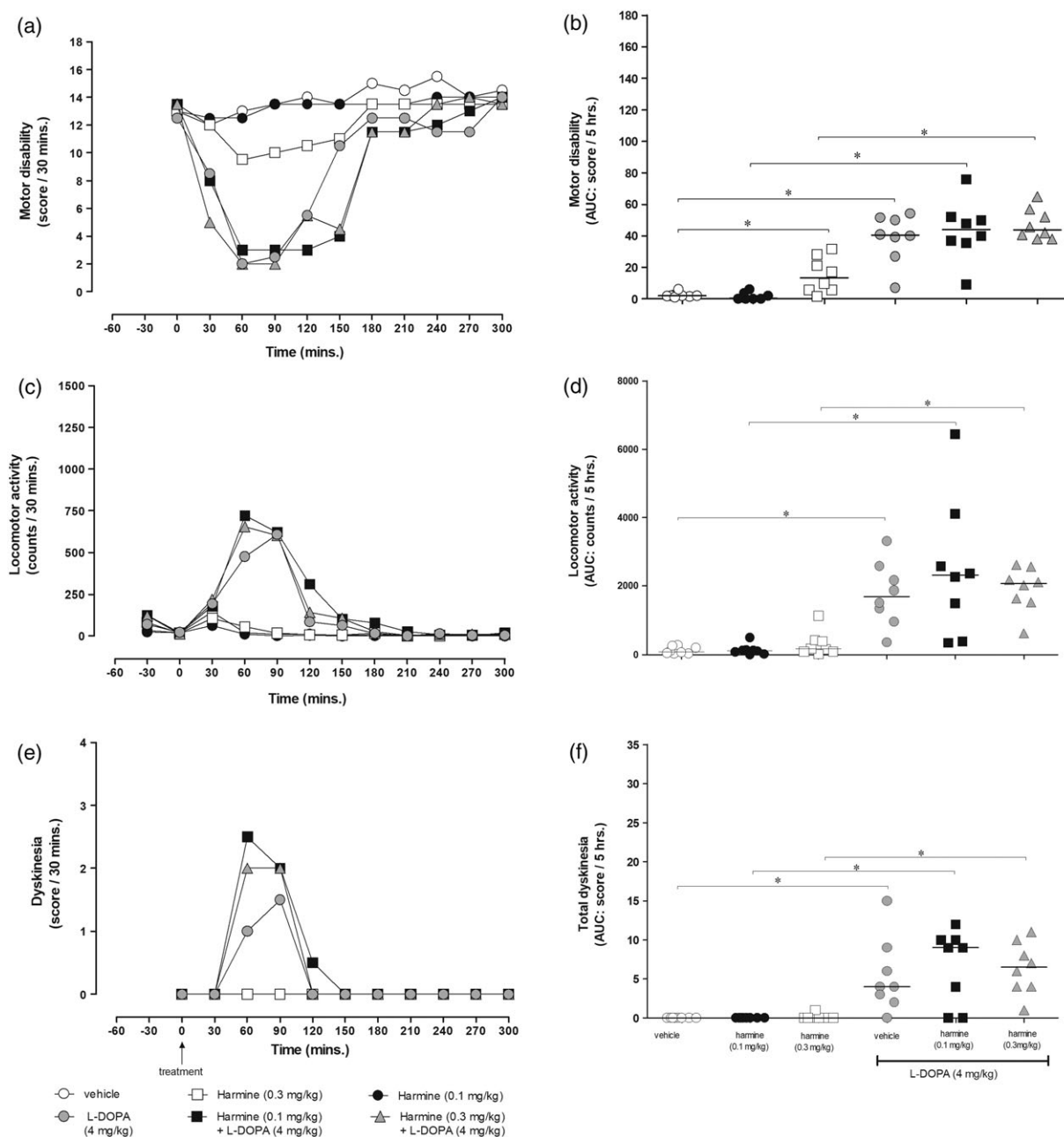


**FIGURE 2** The effect of *Banisteriopsis caapi* extract (28.4, 56.8, and 113.6 mg/kg), L-3,4-dihydroxyphenylalanine (L-DOPA) (7 mg/kg), and vehicle on locomotor activity, reversal of motor disability, and dyskinesia. Treatment was administered at  $T = 0$ , and data are presented as the median values per 30 min for time course data and the median ( $n = 8$ ) and individual values for total (area under curve [AUC]) data. (a) The time course of effect on motor disability following treatment with *B. caapi* alone and in combination with L-DOPA (7 mg/kg, po). (b) The total reversal of motor disability (AUC) following treatment with *B. caapi* alone and in combination with L-DOPA (7 mg/kg, po). \* indicates values that are significantly different ( $p < .0001$ ,  $F$  value = 15.99, degrees of freedom = 7 one-way analysis of variance (ANOVA) for repeated measures and  $p < .05$  Newman-Keuls multiple comparison test). (c) The time course of effect on locomotor activity following treatment with *B. caapi* alone and in combination with L-DOPA (7 mg/kg, po). (d) Total locomotor activity (AUC) following treatment with *B. caapi* alone and in combination with L-DOPA (7 mg/kg, po). \* indicates values that are significantly different ( $p < .0001$ ,  $F$  value = 15.52, degrees of freedom = 7 one-way ANOVA for repeated measures and  $p < .05$  Newman-Keuls multiple comparison test). (e) The time course of effect on dyskinesia following treatment with *B. caapi* and in combination with L-DOPA (7 mg/kg, po). (f) Total dyskinesia (AUC) following treatment with *B. caapi* alone and in combination with L-DOPA (7 mg/kg, po). \* indicates values that are significantly different ( $p < .0001$ ,  $F$  value = 23.55, degrees of freedom = 7, one-way ANOVA for repeated measures and  $p < .05$  Newman-Keuls multiple comparison test)

## 4 | DISCUSSION

Previous studies suggest that extracts of *B. caapi* may possess antiparkinsonian activity based on limited clinical experience in humans (Sanchez-Ramos, 1991; Serrano-Duenas et al., 2001a,

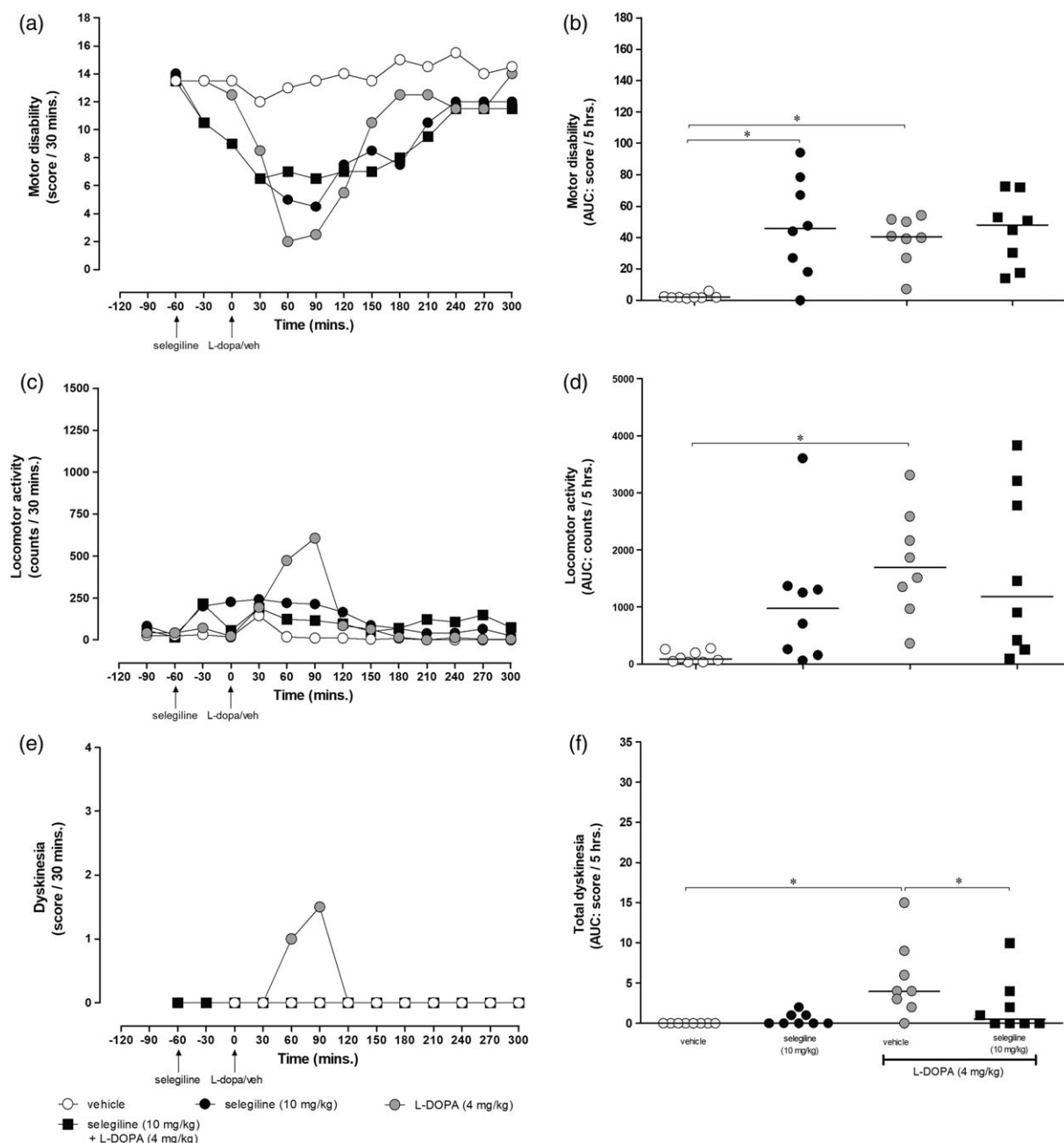
2001b). This activity may relate to the monoamine oxidase inhibitory actions of the  $\beta$ -carboline content of these extracts and to the subsequent effects that their actions have on dopamine metabolism and release in the striatum (Gerardy, 1994; Meneguz et al., 1994). However, alternative mechanisms have been proposed. For example,



**FIGURE 3** The effect of treatment with harmine (0.1 and 0.3 mg/kg sc), L-3,4-dihydroxyphenylalanine (L-DOPA) (4 mg/kg po), and vehicle on locomotor activity, reversal of motor disability, and dyskinesia. Treatment was administered at T = 0, and data are presented as the median values per 30 min for time course data and the median ( $n = 8$ ) and individual values for total (area under curve [AUC]) data. (a) The time course of effect on motor disability following treatment with harmine alone and in combination with L-DOPA (4 mg/kg, po). (b) The total reversal of motor disability (AUC) following treatment with harmine alone and in combination with L-DOPA (4 mg/kg, po). \* indicates values that are significantly different ( $p < .0001$ ,  $F$  value = 44.34, degrees of freedom = 5 one-way analysis of variance (ANOVA) for repeated measures and  $p < .05$  Newman-Keuls multiple comparison test). (c) The time course of effect on locomotor activity following treatment with harmine alone and in combination with L-DOPA (4 mg/kg, po). (d) Total locomotor activity (AUC) following treatment with harmine alone and in combination with L-DOPA (4 mg/kg, po). \* indicates values that are significantly different ( $p < .0001$ ,  $F$  value = 10.81, degrees of freedom = 5 one-way ANOVA for repeated measures and  $p < .05$  Newman-Keuls multiple comparison test). (e) The time course of effect on dyskinesia following treatment with harmine alone and in combination with L-DOPA (4 mg/kg, po). (f) Total dyskinesia (AUC) following treatment with harmine alone and in combination with L-DOPA (4 mg/kg, po). \* indicates values that are significantly different ( $p < .0001$ ,  $F$  value = 20.38, degrees of freedom = 5, one-way ANOVA for repeated measures and  $p < .05$  Newman-Keuls multiple comparison test)

Serrano-Duenas et al. (2001a, 2001b) suggested that the beneficial effects of *B. caapi* were the result of N-Methyl-D-aspartate-receptor antagonist effect of the constituent  $\beta$ -carbolines. In addition, there is evidence that harmine, a component of *B. caapi*, increases striatal dopamine release (Iurlo et al., 2001) and can enhance L-DOPA-

induced stereotypy in normal mice (Pimpinella & Palmery, 1995). However, there is little direct pharmacological evidence for the potential of *B. caapi* extracts to exert an effect on motor function when administered alone, or in combination with L-DOPA, in relation to their potential antiparkinsonian activity in humans. To this



**FIGURE 4** The effect of treatment with selegiline (10 mg/kg sc), L-3,4-dihydroxyphenylalanine (L-DOPA) (4 mg/kg po), and vehicle on locomotor activity, reversal of motor disability, and dyskinesia. Treatment was administered at T = 0, and data are presented as the median values per 30 min for time course data and the median ( $n = 8$ ) and individual values for total (area under curve [AUC]) data. (a) The time course of effect on motor disability following treatment with selegiline alone and in combination with L-DOPA (4 mg/kg, po). (b) The total reversal of motor disability (AUC) following treatment with selegiline alone and in combination with L-DOPA (4 mg/kg, po). \* indicates values that are significantly different ( $p < .0001$ ,  $F$  value = 12.6, degrees of freedom = 3 one-way analysis of variance (ANOVA) for repeated measures and  $p < .05$  Newman-Keuls multiple comparison test). (c) The time course of effect on locomotor activity following treatment with selegiline alone and in combination with L-DOPA (4 mg/kg, po). (d) Total locomotor activity (AUC) following treatment with selegiline alone and in combination with L-DOPA (4 mg/kg, po). \* indicates values that are significantly different ( $p = .0242$ ,  $F$  value = 3.856, degrees of freedom = 3 one-way ANOVA for repeated measures and  $p < .05$  Newman-Keuls multiple comparison test). (e) The time course of effect on dyskinesia following treatment with selegiline alone and in combination with L-DOPA (4 mg/kg, po). (f) Total dyskinesia (AUC) following treatment with selegiline alone and in combination with L-DOPA (4 mg/kg, po). \* indicates values that are significantly different ( $p < .0001$ ,  $F$  value = 11.41, degrees of freedom = 3, one-way ANOVA for repeated measures and  $p < .05$  Newman-Keuls multiple comparison test)

end, we have carried out the first ever examination of extracts of *B. caapi* in the most predictive primate model of drug effect in PD to assess the potential for symptomatic activity. We examined both the effects of *B. caapi* alone and its effects when combined with a

submaximal dose of L-DOPA (4 and 7 mg/kg, po). We compared the effect of the extracts with that of one of the constituent  $\beta$ -carbolines, harmine and with the clinically relevant MAO-B inhibitor, selegiline.



The MPTP-treated primate is highly responsive to L-DOPA treatment, and as expected from previous studies, administration of a submaximal dose (4–7 mg/kg) of the drug reversed the bradykinesia shown by these animals and normalized motor function as indicated by the reversal of motor disability scores. The drug also induced involuntary movements in the animals as they had been primed by prior L-DOPA exposure to exhibit dyskinesia (Pearce et al., 1995). In a similar manner, the higher doses of *B. caapi* administered alone also produced some reversal of motor disability although not to the same extent as L-DOPA (7 mg/kg, po). In contrast to L-DOPA (7 mg/kg, po), there was no increase in locomotor activity and no induction of dyskinesia. This is of interest as increased locomotor activity parallels more closely the induction of dyskinesia than an improvement in motor function related to antiparkinsonian efficacy (Kuoppamäki et al., 2007). This selective effect on motor disability suggests that it may exert an antiparkinsonian action in humans in the absence of the expression of established dyskinesia and would distinguish *B. caapi* extract from the effects of currently used dopaminergic medications. However, when combined with a submaximal dose of L-DOPA (7 mg/kg, po), the effects of the *B. caapi* extract were not additive as no improvement in motor disability was seen over and above that produced by L-DOPA (7 mg/kg, po) alone. Similarly, there was no change in the increased locomotor activity or dyskinesia induction produced by L-DOPA (7 mg/kg, po). These results suggest that the mild antiparkinsonian effect of *B. caapi* may be relevant to early monotherapy in PD but not to the later stages of the disease where adjunct therapy with L-DOPA would be employed. Importantly, the doses of the extract of *B. caapi* were clinically relevant as the lowest dose was equivalent to drinking approximately 150 ml of the original tea in humans. This is consistent with a study in patients with PD where a significant improvement in motor function was reported following a single dose (200 ml) of *B. caapi* tea (Sanchez-Ramos, 1991; Serrano-Duenas et al., 2001a, 2001b).

The question then arises as to whether the modest effects seen with *B. caapi* extracts reflect the monoamine oxidase inhibitory actions of the constituent  $\beta$ -carbolines. The tea was analysed for content of harmine, harmaline, and tetrahydroharmine and found that harmine and tetrahydroharmine, but not harmaline, was present in the tea. This was surprising as previously it has been reported that harmaline is present in the vine; however, levels of harmaline have previously been reported as low (10% of that of harmine; Callaway, Brito, & Neves, 2005). The reason for this difference is not clear but may be due to differences in the extraction processes involved in the analysis. Indeed, in this study, we prepared the *B. caapi* as a tea as it was prepared in water as for earlier clinical trials (Serrano-Duenas et al., 2001a, 2001b), which was freeze-dried and then reconstituted with methanol, whereas in the analysis by Callaway et al. (2005), the samples were solely extracted by methanol.

Administration of the major  $\beta$ -carboline component of *B. caapi*, harmine, at a dose equivalent to that found in the tea extract, produced a modest but significant reversal of motor disability without increased locomotor activity or dyskinesia. This would support a MAOI-based effect of *B. caapi* extracts but cannot exclude the possibility of other pharmacological effect. Harmine has been shown to increase striatal dopamine efflux both in vitro (Schwarz et al., 2003) and in vivo in rats

at equivalent doses (Iurlo et al., 2001). As harmine is a selective inhibitor of MAO-A, and this isoform does not contribute to the metabolism of dopamine in the striatum, it is more likely that this direct effect on DA release explains the reversal of motor disability.

Interestingly, the modest improvement in motor disability was not obvious when harmine was combined with a submaximal dose of L-DOPA (4 mg/kg, po). This is in line with the reported effect of combined treatment with a MAOBI and L-DOPA in PD patients but contradicts findings in 6-OHDA lesioned rats where selegiline potentiated the effects of L-DOPA (Brannan & Yahr, 1995; Macinnes & Duty, 2004; Prat, Perez, Rubi, Casas, & Unzeta, 2000; Skuza, Rogoz, Quack, & Danysz, 1994). However, it would depend on the extent to which the doses of harmine used blocked MAO activity in the brain and the relative selectivity for MAO-A and MAO-B, as it is the latter that is believed to control synaptic dopamine concentrations in the denervated striatum in PD, and prior studies have indicated that harmine has little inhibitory effect on MAO-B (Schwarz et al., 2003).

To assess what changes in motor function would be expected if inhibition of MAO-B activity contributed to the effects of *B. caapi* extracts or harmine, we compared the changes observed to those produced by the selective MAO-B inhibitor, selegiline. In PD, selegiline is used both as monotherapy for its mild symptomatic effects and in later disease as an adjunct to L-DOPA therapy. In the MPTP-treated primate, administration of selegiline reversed motor disability and increased locomotor activity with little or no expression of dyskinesia. These effects were more marked than seen with either *B. caapi* extracts or harmine and suggest that the actions of *B. caapi* and harmine are not wholly dependent on MAO-B inhibition or that they have a weak inhibitory effect at the doses used. This is in line with the evidence suggesting that both harmine and extracts of *B. caapi* have selective inhibitory activity at MAO-A, with little or no effect on MAO-B but that both have modest dopamine releasing properties (Schwarz et al., 2003). As for *B. caapi* and harmine, selegiline administration did not potentiate the effects of a submaximal dose of L-DOPA (4 mg/kg, po) on motor disability and locomotor activity, and although it reduced the peak effects of L-DOPA (4 mg/kg, po), it tended to increase the duration of response, while reducing the expression of L-DOPA-induced dyskinesia. This was unexpected as it does not reflect the clinically accepted effect of selegiline in enhancing dopamine's effect in humans. A MAO-B inhibitor might be expected to potentiate the effects of dopamine formed from L-DOPA at the striatal level (Macinnes & Duty, 2004; Prat et al., 2000; Skuza et al., 1994). Indeed, although selegiline did not alter the overall score for motor disability, the duration of activity, as measured by a score less than 8, was increased by about 1 hr, reflecting the extension of L-DOPA's response and reduced end of dose deterioration reported clinically (Fabbrini, Abbruzzese, Marconi, & Zappia, 2012; Lyytinen, Kaakkola, Gordin, Kultalahti, & Teravainen, 2000). To our surprise, we could find no prior investigation of the symptomatic effects of selegiline in MPTP-treated primates, with the exception of one review that suggests that administration of selegiline increased the antiparkinsonian effects of levodopa and decreased dopamine metabolites (Nomoto, 1995).

Although there is some suggestion that some  $\beta$ -carbolines can be neurotoxic (Haghdoust-Yazdi, Hosseini, Faraji, Nahid, & Jahanihashemi,

2010; Ostergren, Fredriksson, & Brittebo, 2006; Ostergren, Lindquist, & Brittebo, 2007), evidence suggests that harmine is, if anything, neuroprotective with antiinflammatory and antiapoptotic activity (Liu et al., 2017; Zhong, Tao, & Yang, 2015) that could be beneficial in the chronic treatment of PD. In addition, these results in the MPTP-treated primate provide support for the reports of the benefits of *B. caapi* and harmine monotherapy as a mild symptomatic treatment for early PD (Sanchez-Ramos, 1991; Serrano-Duenas et al., 2001a, 2001b), as there was little or no evidence to show that there was any additive or synergistic action in conjunction with L-DOPA that is indicated for mid to late stages of the disease. The effects seen may be due at least in part to the MAO inhibitory actions of some of the constituents of *B. caapi* extract although a direct action on dopamine release cannot be excluded.

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## CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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